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Molecular Characterization of *Mycobacterium tuberculosis* Complex Isolated from Tuberculous Lymphadenitis Patients at Dessie Private Hospitals, Northern Ethiopia

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Abstract
Background: Tuberculosis (TB) is an infectious disease that has major global public health problem. Tuberculous lymphadenitis is among the most common presentations of extra pulmonary tuberculous in developing countries. Although the incidence of Tuberculous Lymphadenitis (TBLN) is rising in different regions of Ethiopia, the incriminated mycobacterium species and strains have been rarely characterized. The present research was aimed to characterize *Mycobacterium tuberculosis* Complex (MTC) and asses associated risk factors.**Methods:** A cross sectional study was conducted from September 2012 up to April 2013 on 90 tuberculous lymphadenitis suspected patients who were visited Dessie Private Hospitals during the study period. Clinical, cytological, Culture, deletion typing and spoligotyping, and interviewer administered questionnaire was used to collect associated risk factors. Data were cleaned, coded and fed into SPSS version 20.0 for analysis in this study. **Results:** Out of 90 fine needle aspirate samples, 33% (32/90) of them were culture positive. Based on the deletion typing of the 32 isolates using region of difference-9 (RD9) showed that all of the isolates were *Mycobacterium tuberculosis*. Further characterization of the 32 isolates using spoligotyping lead to the identification of 28 different strains. Two of these strains consisted of three isolates each while the remaining 26 strains were orphan that consist a single isolate each. Comparison of the 28 patterns with the patterns in the international spoligotype database, SpolDB4 showed that 17 patterns were new spoligotypes. The occurrence of the disease was not associated with age, sex, marital status, occupation, education, previous contacts with TB patients, consumption of raw milk and Bacille Calmette Guerin vaccination (**$p>0.05$**). However, resident area, previous history of treatment with anti-TB drugs and involved lymph nodes were significantly associated with the occurrence of TBLN (**$p<0.05$**). **Conclusion:** this study has shown that 93% of the isolates were orphan which could suggest less frequent transmission of the disease in the study area. Thus, further study on the molecular Epidemiology of the disease should be needed.
Keywords: Dessie Private Hospitals Molecular typing, Northern Ethiopia, Tuberculous lymph adenitis

Background

Tuberculous is the leading cause of death worldwide with a large number of deaths occurring in developing countries. Tuberculous lymphadenitis is among the most common presentations of extra pulmonary tuberculous. TB mainly caused by the member of *Mycobacterium tuberculosis* complex (MTC). Of these species, *M. tuberculosis*, *M. bovis* and *M. africanum* can cause tuberculous lymphadenitis (TBLN) (1).

TBLN is the common form of EPTB and lymph nodes (cervical, axillary and inguinal) are the most commonly involved sites (2). Enlarged lymph nodes are usually firm, painless and may be discrete or matted which varies in size and are non tender (3, 4). There are many factors that accelerate tuberculous infection both in developing and developed countries. Factors like poverty, overcrowding, changing age structure, inadequate health coverage, chronic infections, previous exposure to mycobacterium infections, and neglect and under-funding of TB control programmes (5, 6). The transmission route of EPTB is caused ingestion of raw meat and milk from infected animals (7, 8, 9).

Even though Ethiopia is one of the countries with high prevalence of TBLN, there is limited information about genotypic characteristics of MTC found. But, this information is very vital in order to study phylogenic characteristics of the agents which in turn will provide new insight into the natural history of the disease and it helps to design more effective control measures (10). Thus, the objective the present was to characterize *Mycobacterium tuberculosis* complex and to asses associated risk factors at Private Hospitals, Northern Ethiopia.

Materials and Methods

Study area and design

A cross-sectional study was conducted from November 2012 to May 2013 among at Dessie Private Hospitals in Amhara Regional State, Northern Ethiopia. Dessie town is found 400 km Northern of Addis Ababa, the capital city of Ethiopia. The climate is highland and conducive for animal production. The annual average rainfall is 1800 mm. It is located in latitude and longitude of 9°5°N 36°33°E /9.083°N 36.55°E and an elevation of 2,088

meters. The average temperature was 21°C (12). The Dessie private Referral Hospitals service as referral center for the patients referred to the center from other hospitals, medical centers and private practitioners of Wollo zones and Afar regional state of Ethiopia.

Study Participants

A total of 90 TBLN suspected patients who visited Dessie Private Hospitals were included during the study period. All Fine Needle Aspiration (FNA) samples were collected from area and cultured in LJ media at Aklilu Lemma Institute of Pathobiology (ALIPB). Isolates were stored at -20^{o}C and molecular characterization was performed.

Inclusion and exclusion criteria

Acid Fast Bacilli (AFB) smear positive and/or tentatively clinically diagnosed Tuberculous Lymphadenitis (TBLN) patients who were volunteer to participate in the study and gave their consent or assent, and who were greater than or equal to 5 years old were included in the study. Those TBLN patients who were below 5 years of age who were unwillingness to participate and who already have started anti-TB treatment were excluded.

Specimen collection and processing

FNA samples were taken by physician (pathologist) at the Hospitals with the maximum care and safety and questionnaires were completed at the same time for each patient. All FNA samples were collected using sterile and tightly closed test tubes and kept using phosphate buffer saline at pH 7.2 and +4^{o}C according to (5) recommendations, at the hospitals until transported to ALIPB laboratory for further laboratory tests. The epidemiological and clinical data were also documented at the same time for each patient. Finally, FNA samples were transported to the ALIPB laboratory using ice pack within a week or two for further analysis.

Laboratory Tests: Physical, clinical and cytological examinations were done at Dessie Private Hospitals whereas the Mycobacterium Culture and molecular examinations were conducted at ALIPB.

Mycobacteria culture: All samples were processed and cultured as soon as arrived at the Institute according to the standard methods described by (5). Briefly; the specimen were decontaminated by an equal volume of 4% NaOH, centrifuged at 3000rpm for 15 minutes. Then the supernatant was discarded and the sediment was neutralized with 2N HCl. The neutralization was said to be achieved when the colour of phenol red indicator was changed from red to deep yellow. And the sediment were inoculated into the conventional LJ egg slant medium containing 0.6% sodium pyruvate and glycerol and incubated for at least 6 weeks with weekly observation for the presence of mycobacterial colonies. Microscopic examinations of the colonies were performed by using Ziehl-Neelsen staining method so as to select AFB positive isolates. Heat killed cells were prepared from AFB positive isolates by mixing two loopful of colonies in 200µl distilled water and heating at 80^{o}C for one hour. The same amounts of colonies were kept in glycerol stock at -20^{o}C as backups for future possible related experiments. Finally the heat killed cells were used for molecular characterization.

Molecular Test: RD9 deletion typing and Spoligotyping were done at ALIPB, Addis Ababa University. Heat killed cells of culture positive fine needle aspiration (FNA) samples were investigated by PCR based on deletion typing for the presence or absence of region of difference-nine (RD9) so as to identify *M. tuberculosis* from other species of MTC. For further characterization of the strains, spoligotyping was performed on *M. tuberculosis* isolated by RD9 deletion typing.

Questionnaire survey: The physical and clinical examinations and laboratory investigations were complemented by questionnaire survey. Patients were interviewed using a pre-structured questionnaire in one-visit interview during their sample submission. The focus of the issues in the questionnaire was to identify possible risk factors of acquiring TBLN such as the patients' previous contact with TB patients, consumption of raw milk, Bacille Calmette Guerin vaccination, previous history of treatment with anti-TB drugs and involved lymph nodes.

Data Management and Analysis

The data analysis was conducted using SITVIT2 and SPOTCLUST data base soft ware for strains, and lineages respectively. Clinical data were entered into Microsoft office Excel and processed using SPSS version 20 statistical soft ware. All the data that was collected are entered to MS excel sheet and analyzed by using SPSS version 20. Descriptive statistics was used to determine to assess associated risk factors with the TBLN. In all the analysis, confidence level was held at 95% and $P<0.05$ was set for significance

Ethical Considerations

Before any attempt to collect data, the protocol was approved by Institutional Review Board (IRB) of College of Medical and Health Sciences, Wollega University. Official permission was also obtained from Dessie Referral Private Hospital. The anonymity was warranted for all those records review.

Results

Socio-demographic Characteristics of the Respondents

A total of 90 patients who were suspected of TBLN based on fine needle aspirate cytology positivity (70%) and clinical set up were used for further analysis. Among these patients, females were (48.9%) and were males (51.1%) while the patients were in the young age group (18-30) (58.9%) and rural residents (67.8%). In this

study, of 90 TBLN suspected patients with clinical diagnosis of TBLN, 32 (35.6%) were confirmed as TBLN cases based on combined results of FNA cytology, culture and PCR assay. Resident area and previous history of treatment with anti-TB drugs were significantly associated with the occurrences of TBLN ($p<0.05$). However, the rest of the risk factors were not significantly associated with the occurrence of TBLN ($p>0.05$) (Table1).

In this study associated risk factors were assessed, the major clinical signs and symptoms such as loss of appetite 47.8%, weight loss 35.6% and night sweating 58.9% were observed. Loss of appetite, night sweating and involved lymph nodes were significantly associated with the occurrence of TBLN ($p<0.05$) (Table3).

Mycobacterium Culture: Out of 90 FNA samples, 32 (35.6%) were culture positivity on glycerol containing Lowenstein Jensen (LJ) medium. There was no statistically significant association between associated risk factors and culture results ($P>0.05$)(Table 2).

Region of Difference Based on Deletion Typing: RD9 deletion typing was carried-out on 32 culture positive FNA samples and the results showed that RD9 was present in all of the isolates indicated that the isolates were M. tuberculosis. The majority of the isolates are depicted on (Figure 1).

Spoligotyping: All of the 32 isolates were further characterized by using spoligotyping which lead to the identification of 28 different strains. Two of these strains (SIT50 and SIT393) consisted of three isolates each while the remaining 26 strains were orphan thus, consisting a single isolate each. Comparison of the 28 patterns with the patterns in the international spoligotype database, SpolDB4, showed that 17 of the 28 patterns were not found in the SpolDB4, new spoligotypes while the remaining 11 patterns were reported to the database. Classifying strains on the bases of phylogeny level (lineage) of *M. tuberculosis* using SPOTCLUST software revealed that the strains belonged to Euro-American (EA) ,57.1% (16/28), East-African Indian (EAI), 14.3% (4/28) and Indio Oceanic (IO), 28.6% (8/28) lineages (Table 4).

Discussion

This study identified and characterized *M. tuberculosis* from TBLN patients at Dessie Private Hospitals. In connection, different risk factors were evaluated to see their association with the TBLN. The results of this study showed that there was no statistically significant association of age, sex, occupation, previous contact with TB patients, history of raw milk consumption and BCG vaccination with the occurrence of TBLN. This finding is in agreement with the previous findings (13, 14). However, resident area and previous history of treatment with anti-TB drugs were statistically significantly associated with the occurrence of TBLN. This might be due to lack of awareness on TB, malnourished, the habit of raw milk consumption, inadequate health service and contact with animals of the rural people (15). Although there was previous history of treatment with anti-TB drugs, the people develop the disease. This might related to inadequate treatment, too short administration period, receiving inappropriate dosage so that the organism are getting time to develop resistance to anti-TB drugs or re infection (6,16).

Lower proportion of cases of TBLN was observed in females. This might be due to under-diagnosis or under reporting of TB in females as a result of various social and/or cultural factors, including their consequent impaired access to health care (17). The peak age for TBLN was observed to be between 18 and 30 year, which is consistent with the studies from the United State of America and Europe (18, 19). An explanation for this finding remains unclear, but it is suggested that endocrine factors may play a key role in their behavioral changes in this age group thus increase the chance of exposure to infection. The cervical lymph nodes were the most affected anatomical site in TB confirmed cases. This could associated with the physical closeness of the cervical lymph nodes to the route of infection, as the bacilli can easily picked up by macrophages or dendertic cells which facilitate the transportation of the bacilli in the cervical lymph nodes where they can cause pathology.

In this study, lower FNA cytology and culture were confirmed of the clinically suspected TBLN cases, and this result was in agreement with the reports from Dera by (20) and (14) in three sites (Addis Ababa, Dire Dawa and Bahir Dar). The relatively lower sensitivity could be attributed to the puacibacilary in nature of the FNA samples (21).

The present study confirmed that *M. tuberculosis* was the only isolated Mycobacterium species from the lesions of TBLN in the study area. Although *M. bovis* was highly expected to be isolated from the lesions of TBLN in the study area, no *M. bovis* was isolated. This finding was in agreement with the findings that were reported in different parts of Ethiopia (14, 22) and the absence of *M. bovis* as an etiological agent of TBLN in patients could suggest the minimal role of bovine TB in humans. In contrast to the findings of the present study, studies conducted by (23) isolated *M. bovis* from cases of TBLN cases at Butajira, southern Ethiopia. Similarly, study conducted in Tanzania isolated *M. bovis* from human cervical lymphadenitis cases (24). This might be due to difference in living style and standard, chronic immunosuppressant infections, awareness difference on TB and socioeconomic status.

In this study, 32 isolates of *M. tuberculosis* were typed by spoligotyping and 28 different patterns were identified. Of these, two strains consisted of clusters of isolates while 26 stains consisted of only one isolate each. The study showed that majority of the isolates were orphan (unique pattern) which could suggest less frequent transmission of TBLN in the study area and also suggested that as the patients came from different geographical locations.

The two strains that consisted of clusters of isolates were SIT50 and SIT393 and each consisted of three isolates. The cluster formation indicates exogenous infection resulting from recent transmission and clonal expansion (25, 26).

Of the 28 different strains identified by the present study, SIT37 and SIT52 were reported previously from Ethiopia (22). Although SIT11 (East-African Indian lineage) has been most commonly reported from India, Bangladesh, Sri-Lanka, Malaysia, Indonesia, UK, Denmark, Netherlands, France, New Zealand and USA (27), it was not isolated by the present study. Such findings could suggest the geographic structuring of the clonal population resulting in genetically and phenotypically distinct *M. tuberculosis* population within different parts of the world. Such difference may also explain the geographically variable response to vaccination with *M. bovis* BCG vaccine (28).

In this study, 17 out of 32 spoligotypes possesses heterogeneous spoligotype patterns that could not match with international spoligotype database, new spoligotypes. At the phylogeny level (lineages), results of this study showed that although 'modern' strains of *M. tuberculosis* were the most prevalent, the 'ancient' strains of *M. tuberculosis* such as Indio Oceanic strains were also responsible for the spread of TB. The majority of the strains identified in the present study belonged to the Euro-American lineage. The data confirmed that the old ancestral lineages such as Indio- Oceanic were also circulating in the study population even if in relatively lower proportion (28).

Conclusion

In this study, TBLN is the common form of EPTB and there is a diagnostic challenge in study area. All 32 PCR positive cases of TBLN from the private hospitals were caused by *M. tuberculosis*. Further characterization of the 32 isolates using spoligotyping lead to the identification of 28 different strains. Each of the two strains (SIT50 and SIT393) consisted of three isolates while the remaining 26 strains were orphan thus, consisting a single isolate each. The present study showed that majority of the isolates was orphan which could suggest less frequent transmission of TBLN in the study area and/or the patients came from different geographical locations. Thus, further similar studies should be conducted in different corner of the country in order to fulfill the existing research gap.

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References

1. Singh K, Muralidhar M, Kumar A, Chattopadhyaya T, Kapila K, Singh M, Aharma S, Jain N, Tyagi J. (2000). Comparison of in house Polymerase chain reaction with conventional techniques for the detection of *Mycobacterium tuberculosis* DNA in granulomatous lymphadenopathy. *Journal of Clinical Pathology, 53: 355-361*.
2. Sharma S and Mohan A. (2004). Extra Pulmonary Tuberculosis. *Indian Journal of Medical Research, 120: 4316-4353*.
3. Noussair L, Bert F, Leflon-Guibout V, Gayet N, Nicolas M. (2009). Early Diagnosis of Extra pulmonary Tuberculosis by a New Procedure Combining Broth Culture and PCR. *Journal of Clinical Microbiology, 47(5): 1452-1457*.
4. Alamelu R. (2004). Immunology of tuberculosis. *Indian Journal Medical Reviews, 120: 213-32*.
5. Yang Z, Kong Y, Wilson F, Foxman B, Fowler A, Marrs C, Cave M, Bates J. (2004). Identification of Risk Factors for Extrapulmonary Tuberculosis. *Clinical Infectious Disease, 38: 199-205*.
6. WHO (2008). Global tuberculosis control-surveillance, planning and financing. *WHO report, 1-167*.
7. Nataraj G, Kurup S, Pandit A, Mehta P. (2002). Correlation of fine needle aspiration cytology, smear and culture in tuberculous lymphadenitis: a prospective study. *Brief report, 48: 113-11 6*.
8. De La Rua-Domenech R. (2006). Human Mycobacterium bovis infection in the United Kingdom: incidence, risks, control measures and review of the zoonotic aspects of bovine tuberculosis. *Tuberculosis, 86: 77-109.*
9. LoBue P, Enarson D, Thoen T. (2010). Tuberculosis in humans and its epidemiology, diagnosis and treatment in the United States. *International Journal Tuberculosis Lung Diseases, 14: 1226-1232*.
10. Eyob G, Gebeyehu S, Gashu M, Girma E. (2002). Increase in tuberculosis incidence among the staff working at the Tuberculosis Demonstration and Training centre in Addis Ababa, Ethiopia.A retrospective cohort study (1989-1998). *International Journal of Tuberculosis Lung Disease, 6: 85-88.*
11. WHO (2002). Global Tuberculosis Control. Epidemiology, Strategy and Financing, 1-341.
12. NMSA (National Meteorology Service Agency) (2013): Addis Ababa, Ethiopia
13. Yassin M, Olobo J, Kidane D, Negesse Y, Shimeles E, Tadesse A, Demissie A, Britton S,Harboe M, Aseffa A, Abate G. (2003). Diagnosis of tuberculous lymphadenitis in Butajira, rural Ethiopia. *Scand Journal*

Infectious Dieases, 35(4): 240-243.

14. Iwnetu R, Van Den Hombergh J, Woldeamanuel Y, Asfaw M, Gebrekirstos C, Negussie Y, Bekele T, Ashenafi S, Seyoum B, Melaku K, Yamuah L, Tilahun H, Tadesse Z, Aseffa A. (2009). Is Tuberculous Lymphadenitis Over-Diagnosed In Ethiopia?Comparative Performance of Diagnostic Tests for Mycobacterial Lymphadenitis in a High-Burden Country. *Scand Journal of Infectious Dieases, 41: 462-468.*
15. Gunal S, Yang Z, Agarwal M, Koroglu M. (2011). Demographic and microbial characteristics of extrapulmonary tuberculosis cases diagnosed in Malatya,Turkey. *Bio-Medical and Clinical Public Health, 11: 154-168.*
16. Federal Ministry of Health of Ethiopia (FMoH). (2008). National Tuberculosis and Leprosy Control Program (NTLC) Manual, 4th edn, 5-57
17. Dye C, Watt C, Bleed D, Mehran-Hosseini S, Raviglione M. (2005). Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. *Journal of American Medical Association, 293: 2767-75.*
18. Gonzalez O, Adams G, Teeter L, Bui T, Musser J, Graviss E.(2003). Extra pulmonary manifestations in the large metropolitan areas with a low incidence of tuberculosis. *International Journal Tuberculosis Lung Diseases, 7: 1178-85.*
19. Streeramareddy C, Panduru K, Verma S, Joshi H, Bates, M. (2008). Comparison of pulmonary and extra pulmonary tuberculosis in Nepal - a hospital -based retrospective study. *Journal of Biomedical sciences, 8: 8-30.*
20. Seyuom B. (2005). Characterization of *Mycobacteria*l isolates from Lymph Nodes of Patients with Tuberculous Lymphadenitis in Dera Woreda, North Showa, and Ethiopia. Department of Medical Microbiology,Immunology and Parasitology, Faculty of Medicine, Addis Ababa University.
21. Sinha S, Chatterjee M, Bhattacharya S, Pathak S, Karak K, Mukherjee M. (2011). Diagnostic evaluation of extra pulmonary tuberculosis by fine needle aspiration supplemented with AFB smear and culture. *Journal Indian Medical Association, 101: 588-91.*
22. Beyene D, Bergva I, Hailu E, Ashenafi S, Yamuah L, Aseffa A, Wiker H, Engers H, Klatser P, Sviland L. (2009). Identification and genotyping of the etiological agent of tuberculous lymphadenitis in Ethiopia. *Journal of Infectious in Developing Countries, 3(6): 412-419.*
23. Kidane D, Olobo J, Habte A. (2002). Identification of the causative organism of tuberculous lymphadenitis in Ethiopia by PCR. *Journal of Clinical Microbiology, 40: 4230-4234.*
24. Hasan Z, Tanveer M, Kanji A, Hasan O, Ghebremichael S, Hasan R. (2006). Spoligotyping of *Mycobacterium tuberculosis* Isolates from Pakistan Reveals Predominance of Central Asian Strain 1 and Beijing Isolates. *Journal of Clinical Microbiology, 44 (5): 1763-1768.*
25. Sebastein G, Riemer K, Van T, Kato-Maeda M, Bouke C, Jong D, Narayanan S, Nicol M, Niemann S, Kremer K, Gutierrez MC, Hilty M, Hopewell P, Small P. (2006). Variable host-pathogen compatibility in *Mycobacterium tuberculosis. National Academic Science, USA, 103 (8): 2869-2873.*
26. Filliol J, Driscoll R. (2006). Snapshot of moving and expanding clones of *Mycobacterium tuberculosis* and their global distribution assessed by spoligotyping in an international study. *Journal of Clinical Microbiology, 41(5): 1963-1970.*
27. Baker L. and Brown T. (2004). Silent nucleotide polymorphisms and phylogeny for *Mycobacterium tuberculosis. Emerging Infectious Diseases, 10 (9): 1568-1577.*
28. Palomino J, Sylvia C, Viviana R. (2007). Tuberculosis 2007: From basic science to patient care. TuberculosisTextbook.com
29. WHO (2011). Global tuberculosis control. Geneva, WHO report 35-6 wide- Fine needle aspiration in the diagnosis of tuberculous lymphadenitis in Africa. AIDS, 5:213-98.

Table1: Socio-demographic Characteristics of the study subjects and their respective FNA cytology results

Risk Factors		Non TBLN	TBLN	Frequency	P-values
Sex	Male	15	31	46	0.58
	Female	12	32	44	
Age Category in years	18-30	17	36	53	0.47
	31-45	5	19	24	
	>45	5	8	13	
Marital Status	Single	14	20	34	0.08
	Married	13	43	56	
Resident area	Urban	13	16	29	**0.03**
	Rural	14	47	61	
Education	Literate	18	28	46	0.05
	Illiterate	9	35	44	
Occupation	Pastoralist	4	17	21	0.28
	Non Pastoralist	23	46	69	
Consumption of raw milk	Yes	5	22	27	0.12
	No	22	41	63	
Previous contact with TB Patients	Yes	6	25	31	0.11
	No	21	38	59	
Contact with whom?	Outside family	26	51	77	0.18
	Family	1	12	13	
History of treatment	Yes	1	16	16	**0.02**
	No	26	15	74	
BCG vaccination	Yes	2	4	6	0.59
	No	25	59	84	

Table2: Association between socio-demographic characteristics and their culture results

Risk Factors		Culture		P-values
		Positive	Negative	
Sex	Male	15	31	0.55
	Female	17	27	
Age Category in years	18-30	18	35	0.93
	31-45	9	15	
	>45	5	8	
Marital Status	Single	11	23	0.62
	Married	21	35	
Residence area	Urban	9	20	0.54
	Rural	23	38	
Education	Illiterate	18	26	0.30
	Literate	14	37	
Occupation	Pastoralist	7	14	0.80
	Non Pastoralist	25	44	
Consumption of raw milk	Yes	8	19	0.44
	No	24	39	
Previous contact with TB Patients	Yes	11	20	0.99
	No	21	38	
Contact with whom?	Outside family	25	52	0.14
	Family	7	6	
History of treatment	Yes	6	10	0.86
	No	26	48	
BCG vaccination	Yes	3	3	0.36
	No	29	55	

Table3: Clinical variables and their respective FNA cytology results

Clinical variables		Non TBLN	TBLN	P-values
Cough	Yes	6	25	0.11
	No	21	38	
Fever	Yes	6	19	0.44
	No	21	44	
Loss of Appetite	Yes	17	43	0.63
	No	10	20	
Loss of weight	Yes	5	32	**<0.001**
	No	22	31	
Chest pain	Yes	8	28	0.19
	No	19	35	
Shortness of breathing	Yes	2	4	0.59
	No	25	59	
Night sweatiness	Yes	7	53	**<0.001**
	No	20	10	
Fatigue	Yes	11	18	0.26
	No	16	45	
Lymph nodes involved	Cervical	11	27	**0.01**
	Axillary	10	7	
	Inguinal	7	28	

Table4. Spoligotype patterns of *M. tuberculosis* isolates identified in the study area

Sample's Code	SIT	No. of isolates	Lineages	Ancestral/ Modern
SE26	26	1	East-African Indian	Ances
SE123	289	1	East-African Indian	Ances
Se111	New	1	Euro-American	Modern
Se112	New	1	Euro American	Modern
Se126	New	1	Euro American	Modern
Se118	New	1	Indio Oceanic	Ances
Se102	21	1	East-African Indian	Ances
SE121	New	1	Indio Oceanic	Ances
SE06	New	1	Euro American	Modern
Se105	New	1	Indio Oceanic	Ances
Se41	393	3	Euro American	Modern
Se23	New	1	Euro American	Modern
Se129	50	3	Euro American	Modern
Se21	New	1	Euro American	Modern
Se38	New	1	Euro American	Modern
Se46P	929	1	Euro American	Modern
SE114	New	1	East African Indian	Ances
Se 29	New	1	Indio Oceanic	Ances
SE46G	New	1	Indio Oceanic	Ances
Se128	53	1	Euro American	Modern
SE134	334	1	Euro American	Modern
Se42	52	1	Euro American	Modern
Se119	New	1	Indio Oceanic	Ances
SE20	New	1	Indio Oceanic	Ances
Se39	New	1	Euro American	Modern
Se117	302	1	Euro American	Modern
Se44	37	1	Euro American	Modern
Se121	New	1	Indio Oceanic	Ances

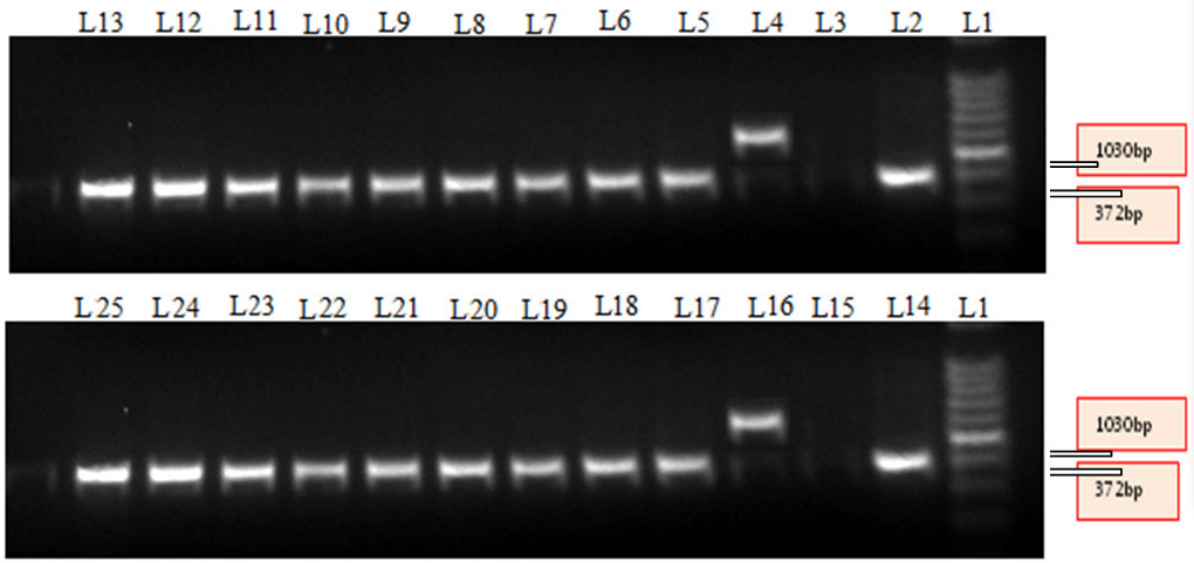

Figure 1: Selected gels showing the results of RD9 deletion typing
Lane (L) 1=100bp ladder (*M. tuberculosis* marker, Lane2= H37Rv (positive control), Lane3= Qiagen H20 (negative control), Lane4= *M. bovis* (positive control), Lane5-26=FNA samples Present for *M. tuberculosis.*

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